REMARKS

Favorable reconsideration is respectfully requested in view of the foregoing amendments and the following remarks.

I. EXAMINER INTERVIEW

Applicants thank Examiners Moore and Nashed for the personal interview on September 9, 2005.

During the interview, the Examiners indicated that an adequate English translation of the priority document is required to replace the specification of record. The Examiners requested a specification that more precisely sets forth and defines the subject matter of the claimed invention.

Pursuant to the Examiners' request and in response to the objection to the specification, enclosed herewith is an adequate English translation of the priority document as a substitute specification. The specification has been amended to reflect this translation.

During the interview, it was agreed that the disclosure and the state of the art establish a scope for an indicator enzyme in the probe other than a luciferase enzyme and a green fluorescent protein.

Also, it was agreed that the cited prior art references (Remy and Wu) do not suggest the alterations disclosed in the specification to the amino acid sequences of the luciferase and green fluorescent protein.

Lastly, the Examiners provided the Applicants with copies of the following references: (1) Ozawa et al., Current Opinion in Chemical Biology, vol. 5, pp. 578-583 (2001); and (2) Ozawa et al., Anal. Chem., vol. 72, pp. 5151-5157 (November 1, 2000). The inventors of the instant application are co-authors of the references. It was indicated that these references help explain the nature of invention and the state of the art, and thus, they may be useful in preparing the translation of the priority document.

II. CLAIM STATUS & AMENDMENTS

The specification at page 18, lines 6-13 is amended to delete the subject matter added per the previous response. Support can be found in the originally filed specification.

An adequate English translation of the priority document is submitted herewith as a substitute specification. The specification and abstract have been carefully reviewed and revised in order to correct grammatical, idiomatic and translational errors in order to aid the Examiner in further consideration of this application. The amendments to the specification and abstract are incorporated in the attached substitute specification and abstract. No new matter has been added.

Also attached herewith is a marked-up version of the substitute specification and abstract illustrating the changes made to the specification and abstract.

Also attached herewith is a Translator's Declaration. This Declaration certifies that the attached substitute specification is an accurate translation of the priority document, PCT/JP00/09348.

Claims 1, 3 and 6-11 were pending in this application when last examined, and stand rejected.

Claim 1 is amended to replace "A probe for analyzing protein-protein interaction between two proteins" with "A set of probes for analyzing protein A - protein B interaction. . ." Claim 1 is also amended to clarify the relationship between the two probes and the two proteins. Specifically, claim 1 is amended to define the structural relationships of the components in probes "a" and "b" as follows:

"a": N-N-half indicator-C+ N-N-half intein-C+ connection site for protein A;

"b": connection site for protein B+ N-C-half intein-C+ N-C-half indicator-C.

Support for these structures can be found in Fig. 4 and Fig. 7. These structures satisfy the Examiner's requirement from page 8, the last 2 lines to page 9, lines 1-2 of the Office Action.

Support for "protein A", "protein B" and "analyzing protein A – protein B interaction" in the amended claims can be found in the specification, for instance, at page 10, lines 14-24, wherein it is disclosed that:

> The two probes a (1a) and b (1b) are separately connected to the respective proteins A (4a) and B (4b) for which interaction is to be analyzed, to detect the protein A-protein B interaction. Hence, when protein A (4a) and protein B (4b) each connected to probe a (1a) and probe b (1b), respectively, coexist (I) and when the two proteins (4a, 4b) interact with each other (II), the intein (2) is excised through splicing (III). As a result, the labeled protein sites (3a, 3b) bonded to the intein (2) are ligated (IV), thereby enabling the confirmation of the protein-protein interaction through detection of the signal resulting from the labeled protein (3).

Based on this disclosure, it is clear that the terms 'protein A" and "protein B" and "protein A – protein B interaction" refer to an interested protein and a target protein and the interaction between an interested protein and a target protein.

Kindly note that the term "labeled protein" in claim 1 is replaced with "indicator protein." In the 2nd paragraph on page 9 of the Office Action, it was requested that the term "labeled" be replaced with a functionally descriptive term since the specification does not disclose that any radiant indicator protein is "labeled". Similarly, the specification has been revised to replace "labeled protein" with "indicator protein" as this term is a more precise translation from the description of the original Japanese priority document. It is respectfully submitted that the amended term "indicator protein" satisfies the Examiner's requirement.

Claims 6-9 are amended to recite the proper antecedent basis for "The set of probes for analyzing protein A - protein B interaction" to be consistent with the amendment to claim 1.

Claim 11 is cancelled without prejudice or disclaimer thereto. Applicants reserve the right to file a continuation of divisional application on any cancelled subject matter.

Claims 12 and 13 are newly added.

New claim 12 is directed to an expression vector, which co-expresses probe "a" and probe "b" as a fusion polypeptide, respectively. This is based on Figs. 4 and 7 of the disclosure. Support for new claims 12 can also be found in original claim 11.

New claim 13 is an analysis method using the expression vector of claim 12. Support can also be found in original claim 11.

Therefore, no new matter has been added by this amendment.

Claims 1, 3 and 6-10, 12 and 13 are pending upon entry of this amendment.

III. NEW MATTER OBJECTION

On pages 2-3, the amendment filed March 7, 2005 is objected for adding new matter to the specification on page 18.

It is respectfully submitted that the present amendment overcomes this objection.

As noted above, the subject matter added at page 18, lines 6-13 per the previous response has been deleted.

In view of the above, the new matter objection is untenable and should be withdrawn.

IV. OBJECTION TO THE SPECIFICATION

On pages 3-4, the specification remains objected to for containing grammatical errors, unclear/inexact terminology and inconsistencies.

An adequate translation has been submitted as a substitute specification and the specification has been appropriately amended.

In view of this amendment, the objection to the specification is untenable and should be withdrawn.

V. CLAIM OBJECTIONS

On pages, claims 1 and 11 were objected to for containing minor informalities.

It is respectfully submitted that the present amendment overcomes this objection.

As noted above, claim 1 is amended to clarify the relationship between the two probes and the two proteins. Claim 11 is cancelled and the objection to claim 11 should be cleared by new claims 12 and 13.

In view of the above, the objection to claims 1 and 11 is untenable and should be withdrawn.

VI. ENABLEMENT REJECTION

On pages 5-8, claims 1,3 6-8, 10 and 11 were newly rejected under 35 U.S.C. § 112, first paragraph, on the basis that the specification is enabling for a probe comprising a labeled protein which is a firefly luciferase divided at amino acids 437 and 438 into N-terminal and C-terminal portions, but not for the probes of claims 8 and 9 comprising a generic luminescent enzyme nor the probes of claims 6 and 7 comprising an indicator which is a green fluorescent protein, such as native EGFP. The Examiner contends that neither the prior art of record or the instant disclosure teach suitable locations for dividing luminescent enzymes and flourescent proteins with intein components to ensure proper functioning of such proteins after reconstitution.

This rejection is respectfully traversed as applied to the amended and new claims.

The test of enablement is whether one reasonably skilled in the art could make or use the invention based on the disclosure in the specification coupled with the knowledge in the art without undue experimentation. The fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation. The test is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue. See M.P.E.P. § 2164.01.

During the interview, it was agreed that the disclosure and the state of the art establish a scope for an indicator enzyme in the probe other than a luciferase enzyme and a green fluorescent protein.

In this regard, kindly note that the specification at pages 14, lines 15-18 discloses that "[f]or the split N– and C- terminals of the luciferase to give no fluorescence when separated and restore its emission activity after its two terminals are recombined , the luciferase must be split so that its activity center is divided into two." At page 14, lines 18-25 and at page 15, lines 1-3, the specification further describes the known structure of the luciferase enzyme based on the prior art of record. For instance, as disclosed in the specification, the luciferase enzyme is folded into two domains that sandwich a broad activity center region and "it is desirable to that the enzyme luciferase is split into two at the flexible side through which the two domains are linked to each other."

At page 15, lines 3-15, the specification even identifies specific amino acid mutations to enable efficient protein splicing. For instance, the specific location of the luciferase, between position 437 and 438, is an example for dividing the luciferase between the two domains.

Attached herewith are abstracts for journal references providing further evidence of the structure of the various known vectors, plasmids and indicator proteins, such as luciferases and green fluorescent proteins, and their corresponding amino acid sequences.

Based on this disclosure and the state of the art, it is respectfully submitted that the skilled artisan could select the suitable location for dividing a generic luminescent enzyme without undue experimentation.

Similarly, as to the fluorescent proteins of claims 6 and 7, it is respectfully submitted that the skilled artisan could also select a suitable site for dividing the fluorescent proteins into two parts without undue experimentation. In this regard, attached herewith are journal references providing further evidence of the structure of the various known fluorescent proteins and their corresponding amino acid sequences. The structure of fluorescent proteins was well known in the art at the time of invention as evidenced by the attached references.

The specification also provides specific examples of the construction of a construct of the claimed invention. For instance, the construction of the plasmid of the present invention

(pGEX_NVC) is based on the Example in the specification, whereby pGEX_NVC is constructed from commercial pGEX vectors (e.g. Amersham Biosciences Corp.: http://www1.amershambiosciences.com/aptrix/upp01077.nsf/Content/Products?OpenDocument&parentid=27459701&moduleid=38859). The construction and the sequence of each commercial pGEX vector are known to the public, and the "BamH I" restriction site is the only site in the commercial pGEX vectors.

As shown in Fig. 4, pGEX_NVC is constructed by: (1) preserving the GST region of a commercial pGEX vector; (2) linking the N-terminus of mutant EGFP to the C-terminus of the BamH I restriction site of the commercial pGEX vector; and (3) inserting an intein such as "VDE" into a sequence of mutant EGFP.

Accordingly, a base vector for the pGEX_NVC is obtained from the "commercial pGEX vectors series", and the construction process of pGEX_NVC is shown in Fig. 4.

Also, with regard to "dividing EGFP by an intein", it is respectfully submitted that this is adequately explained in the specification at page 14 line 18, Fig. 4, etc.

Lastly, as noted above, claim 1 is amended to define the structural relationships of the components in probes "a" and "b" as follows:

"a": N-N-half indicator-C+ N-N-half intein-C+ connection site for protein A;

"b": connection site for protein B + N-C-half intein-C+ N-C-half indicator-C.

Support can be found in Fig. 4 and Fig. 7 of the disclosure.

Thus, based on the disclosure and the state of the art, it is respectfully submitted that the skilled artisan could make and use the claimed probes utilizing an indicator enzyme other than a luciferase enzyme and a green fluorescent protein without undue experimentation. Again, this was agreed to during the interview.

Thus, for the reasons set forth above, the enablement rejection of claims 1,3 6-8, 10 and 11 under 35 U.S.C. § 112, first paragraph, is untenable and should be withdrawn.

VII. INDEFINITENESS REJECTION

On pages 8-10, claims 1, 3, 6-8, 10 and 11 remain rejected under 35 U.S.C. § 112, second paragraph, for failing to describe the structural relationship/orientation of the intein and indicator protein components of probes (a) and (b).

It is respectfully submitted that the present amendment overcomes this rejection as applied to the amended and new claims.

Amended claim 1 clarifies the ordered structural relationship of probes "a" and "b" as supported by the disclosure at page 10, lines 10-24. Furthermore, amended claim 1 requires hat probe "a" and probe "b" separately connect to respective target proteins for which protein interaction is two be analyzed, and when the target proteins interact with each other, the inteins are excised through splicing. This amendment is supported the structures based on Fig. 4 and Fig. 7.

With regard to the term "linked", kindly note that this term has been removed from the claim.

Thus, in view of the above, the rejection of claims 1, 3, 6-8, 10 and 11 under 35 U.S.C. § 112, second paragraph, is untenable and should be withdrawn.

VIII. OBVIOUSNESS REJECTION

Claims 1, 6, 10, and 11 were newly rejected under 35 U.S.C. § 103(a) as obvious over Remy et al. (PNAS, USA, Vol. 96, pp. 5394-5399 (1999)) and Wu et al. (PNAS, USA, Vol. 95, pp. 9226-9231 (1998 (Wu 1998a)) in view of Wu et al. (Biochimica et Biophysica Acta, Vol. 1387, pp. 422-432 (1998) (Wu 1998b)). See pages 10-12 of the Office Action.

This rejection is respectfully traversed as applied to the amended and new claims.

Amended claim 1 is directed to a set of probes for analyzing protein A - protein B interaction, which comprises: (1) probe "a" comprising a N-half of an intein polypeptide and a N-half of an indicator protein, wherein the N-half of the indicator protein is connected at the

N-terminal end of the N-half of the intein polypeptide, and the C-terminal end of the N-half of the intein polypeptide a site for connecting protein A; and (2) probe "b" comprising a C-half of the intein polypeptide and a C-half of the indicator protein, wherein the C-half of the indicator protein is connected at the C-terminal end of the C-half of the intein polypeptide, and the N-terminal end of the C-half of the intein polypeptide is a site for connecting protein B.

Amended claim 10 is directed to a method for analyzing protein A - protein B interaction by using the set of probes of claim 1, which comprises: (1) connecting protein A with probe "a", and connecting protein B with probe "b"; (2) introducing probe "a" and probe "b" in a system; and (3) detecting the interaction of protein A with protein B by measuring a change of the signal from the indicator protein that is a fusion protein consisting of the N-terminal half of the indicator protein and the C-terminal half of the indicator protein.

Amended claim 14 is directed to vector for expressing the set of probes.

The cited prior art references fail to disclose or suggest such constructs. Furthermore, one feature of the invention is that the indicator protein gives the signal by intein which is cut out. The cited references fail to disclose or suggest this.

Furthermore, the present invention does not have to use various substrates. However, Remy requires the use of various substrates to be able to apply only to the cell appropriately designed and to analyze the cell. Furthermore, it is rapamycin dependent.

Thus, in view of the above, the rejection of claims 1, 6, 10, and 11 under 35 U.S.C. § 103(a), is untenable and should be withdrawn.

CONCLUSION

In view of the foregoing amendments and remarks, the present application is in condition for allowance and early notice to that effect is hereby requested.

If the Examiner has any comments or proposals for expediting prosecution, please contact the undersigned attorney at the telephone number below.

Respectfully submitted,

Yoshio UMEZAWA et al.

By: _____

Jay F. Williams
Registration No. 48,036
for
Warren M. Cheek, Jr.
Registration No. 33,367
Attorneys for Applicants

WMC/JFW/akl
Washington, D.C. 20006-1021
Telephone (202) 721-8200
Facsimile (202) 721-8250
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ATTACHMENTS

1. Substitute specification and abstract, both clean copy and marked-up copy;

2. Translator's Declaration – certifying the translation to be accurate; and

3. Abstracts for journal references providing evidence of the structure of the various known indicator proteins.